SPECIAL COMMUNICATION

The following extended abstracts were presented at a Research Initiatives in Vascular Disease Symposium, *Vascular Gene Transfer: Models of Disease and Therapy,* sponsored by the National Heart, Lung, and Blood Institute of the National Institutes of Health, and the Lifeline Foundation of the Society for Vascular Surgery and the North American Chapter, International Society for Cardiovascular Surgery on March 7-8, 1996, in Bethesda, Md.

OVERVIEW ON GENE TRANSFER

RECOMBINANT GENE TRANSFER: SCIENTIFIC INSIGHTS, DISEASE PATHOGENESIS, AND ITS POTENTIAL TO TREAT HUMAN DISEASE

During the past decade, significant progress has been made in understanding the genetic basis for congenital and acquired diseases. Concurrently, there have been substantial advances in knowledge about the scientific basis for genentic development and its role in the disease process. This increased comprehension about the underlying mechanisms has led to the evolution of gene transfer techniques to attempt correction of defective gene function. There are several critical factors in the development of strategies for gene therapy. These include the type of vector used, the design of the vector, and the method of delivery. Many different approaches for employing gene transfer for the treatment of disease have been developed and evaluated. Until recently, viral vectors (e.g., retroviral, adenoviral and adeno-associated virus [AAV]) were the predominant method for delivering genes into cells because of their relatively high transduction efficiency. In response to growing concerns regarding the safety of viral vectors, considerable effort is now being expended to optimize nonviral delivery systems (naked DNA, DNA-liposome complexes, and particle-mediated delivery of DNA coated to inert beads). These endeavors have succeeded in providing a safer and viable alternative for a number of applications.[1]

Ex vivo delivery has been the most commonly tested method for gene transfer in the past because it is often difficult to transduce target cells or tissue inside the patient; however, there are significant disadvantages to performing gene therapy in this manner. Ex vivo delivery necessitates that cells become established in cultures in the laboratory and survive the manipulations required for the transduction and subsequent selection of transformed cells before being reintroduced into the patient. Thus it subjects the cells to selection and different growth conditions from those that are exerted in vivo and requires that cell lines be established for each malignancy. The adaptability of in vitro gene transfer approaches to human disease is therefore more difficult. There has recently been progress with in vivo gene transfer using nonviral vectors. Direct injection and catheter-based delivery of genes are two methods for effecting transduction of cells, although the scope of their utility is still limited.

The development of appropriate vectors is another critical factor in providing effective gene therapy. In addition to containing the gene of interest, the vector must incorporate sequences that maximize the transcription and translation of the gene. Vector design and testing systems have improved considerably in recent years. Among the recent advances are the incorporation of multiple genes into vectors and the development of vectors that can potentially cause the destruction of the transduced cells when specific drugs are administered, ensuring that gene expression is self-limiting.

Gene therapy techniques have been tested for the treatment of a number of inherited genetic disorders in which there are mutations in the coding or regulatory sequences. Attempts have been made to incorporate the adenosine deaminase (ADA) gene into human T lymphocytes to complement ADA deficiency, which leads to immune deficiency. Another study has used the LDL receptor gene in an effort to treat patients who are deficient in this receptor, resulting in hypercholesteremia. Studies are ongoing to develop gene therapies for cystic fibrosis using adenoviral vectors, as well as hemophilia (factor IX and VIII), phenylketonuria (phenylalanine hydroxylase, thalassemia (β-globin) and sickle cell anemia (β-globin).[2] In general, although the genetic lesions in these diseases are well understood, progress toward treatment has been limited by the need to express the gene in the appropriate tissues and regulate it appropriately and at sufficient levels to impact on these diseases.

Gene therapy is now being developed for the treatment of a variety of diseases in addition to those caused by genetic defects. These conditions include cancer, cardiovascular disease, and infectious diseases. Important advances have been made in the use of gene therapy for the treatment of cancer. One approach focuses on enhancing the immune response against tumors by the introduction of genes directly into tumor cells in vivo. The immune system is designed to provide protection against foreign agents. When the host is unable to recognize an organism or substance as foreign, the system fails to provide adequate protection, and the safety of the host is compromised. One method that is currently being tested is to administer a DNA/liposome complex encoding for a class I MHC antigen directly into the tumor in vivo by direct injection or catheter-mediated delivery. When this gene is expressed on the cell surface of HLA-B7 negative individuals, it stimulates an immune reaction. It has been demonstrated that when this occurs, the host begins to recognize previously undetected antigens expressed by the cancer cells.[3,4]

The potential use of gene therapy to alter the course of infection by HIV is advancing through the testing of several different approaches.[5] These include decoys, antisense oligonucleotides, ribozymes, and intracellular immunization. Decoys are a method of causing cells to overexpress binding sequences.[6] When sequences specific to HIV are chosen, it is possible to reduce replication of the virus.[7-9] Antisense oligonucleotides, which bind to functional sequences in the HIV genome, are another method for treating HIV through genetic modulation. Their administration potentially leads to disruption in HIV replication.[10] A third gene therapy method is the use of ribozymes, which are a more reactive form of oligonucleotides, based on RNA catalytic function. These agents bind to the sequence, then cleave the nucleic acids from the structure. In this way, the virus is inactivated. Another area that is being pursued for the treatment of HIV is the use of genes encoding for transdominant negative proteins to interfere with the activation and regulation of HIV. This objective can be accomplished through the retroviral and nonviral delivery of the gene into CD4+ cells from HIV infected individuals. This approach is

currently being tested using Rev M10, a mutant form of the transdominant form of Rev.

Both cell-mediated and direct gene transfer have been used in the optimization of gene therapies for vascular diseases.[11] Cell-mediated gene transfer is an ex vivo procedure in which the vector is introduced into endothelial or smooth muscle cells that have been harvested from an artery or vein and are maintained in cell culture. These cells are then delivered through a catheter into prespecified areas of an artery, where they establish themselves. Investigations of vector delivery using replication-defective adenoviral vectors and DNA liposome complexes have been undertaken. The transfection efficiency provided by adenoviruses is higher than that obtained through other methods, but it is not yet known whether this expression elicits the desired reaction without causing toxicities. Improved nonviral vectors such as DNA liposome complexes may provide an alternative approach. Recently, an adenoviral vector expressing herpesvirus thymidine kinase was introduced in vivo into balloon-injured porcine arteries. These cells responded to treatment with the nucleoside analog, ganciclovir, decreasing intimal hyperplasia.[12] Additional studies are now being conducted to optimize the vectors and delivery system. Several other gene products that target cell proliferation, angiogenesis, or contractility have also shown promise in different animal models.

Much progress has been made in understanding the role of specific genes in the pathogenesis of human disease. Gene therapy is now being tested in clinical studies for diverse medical problems. There is significant potential for this area of research to impact on both the understanding and treatment of a variety of human diseases.

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ANIMAL MODELS OF DISEASE

LIPID DISORDERS AND ATHEROSCLEROSIS IN GENETICALLY MODIFIED MICE

It is now generally accepted that the lesions of atherosclerosis begin as a result of some form of insult to the lining endothelial and smooth muscle cells of the artery wall. This insult can relatively rapidly lead to the development of a specialized, chronic inflammatory response, characterized by the presence of peripheral blood monocytes and T lymphocytes. In individuals with hyperlipidemia, transport of lipoproteins by the endothelial cells from the plasma into the artery wall may result in modification of some of these lipoproteins and, in part, to their oxidation. These modified lipoproteins may, in turn, injure the overlying endothelial cells, resulting in continuing adherence of monocytes and T lymphocytes and to the development of chemotactic factors within the artery wall that draw these leukocytes between lining endothelial cells into the subjacent intima.

Studies in human beings, nonhuman primates, rabbits, swine, and, most recently, hyperlipidemic, transgenic ApoE-deficient mice demonstrate that one of the earliest observable changes within the artery is the increased adherence of monocytes and T lymphocytes to the endothelium. These cells adhere to the endothelium in clusters that appear to be localized throughout the arterial tree. Many of these adherent leukocytes are attached to the endothelium at sites of flow alteration, such as branches and bifurcations, as well as at the flow dividers within various parts of the artery. Before the appearance of these adherent leukocytes, particulate matter can be seen in the region between the endothelium and its underlying basement membrane. This particulate matter has the appearance of membranous whorls, as well as of lipoprotein particles, and has been described as liposome-like material. Thus there is evidence from human surgical and autopsy material and from specimens of experimental animals that monocyte and T lymphocyte adherence is preceded by the deposition of lipoid material beneath the endothelial cells. This lipoid material may somehow induce the formation of specific cell-surface adhesive glycoproteins on the surface of the endothelium, which could then lead to binding and attachment of monocytes and T cells.

The chemotactic attraction of these leukocytes into the artery leads to their localization subjacent to the endothelium and to the conversion of many of the subendothelial monocytes into macrophages or scavenger cells. These macrophages can actively bind and internalize the modified (oxidized) lipoproteins that may be present within the intimal space, become lipid laden, and take on the appearance of foam cells. Foam cell formation may lead to activation of the macrophages and to expression of genes for a series of cytokines and growth-regulatory molecules. Cytokines that may be expressed by the macrophages include interleukin-1 (IL-1) and tumor necrosis factor (TNF), whereas the T lymphocytes may express the gene for interferon (IFN). In addition to the formation of cytokines, the macrophages have the capacity to express genes for platelet-derived growth factors A and B (PDGF-A and PDGF-B), insulin-like growth factor 1 (IGF-1), heparin-binding EGF-like growth factor (HB-EGF), and transforming growth factor β (TGFβ), as well as numerous others. The expression of these genes usually leads to their translation and, ultimately, to the possible secretion of these growth factors. Smooth muscle cells in the subjacent media of the artery wall contain receptors for PDGF,